Inoculum Preparation Affects In Vitro Gas Production During Early Fermentation

D.R. Mertens

Introduction

Gas production during microbial fermentation may be a useful marker for estimating the rate and extent of digestion of feeds. Measuring gas production instead of the disappearance of substrates such as fiber or dry matter has several advantages. It measures the sum total of all fermentation and estimates the total energy contribution of the rumen to the cow from fiber, soluble carbohydrates and proteins. It is not contaminated by microbial residues like residual dry matter, which complicates the use of dry matter residues to estimated digestion kinetics. Gas production is much easier to measure than fiber disappearance because serial samples do not have to be analyzed individually.

Recent work indicated that the source of inoculum had a significant impact on in vitro results. Preliminary experiments suggested that the method for preparing the inoculum also affected results. Initally, inoculum was prepared by blending ruminal solids with chilled in vitro media to detach microbes that cling to particles. Strained ruminal fluid was combined with the strained blended media to produce the inoculum. However, this inoculum yielded relatively high gas production for blanks (in which no sample material is included), and cooling the inoculum probably slowed initial fermentation rates. The objectives of these studies were to determine if removing solids by centrifugation would reduce blank gas production and affect gas production kinetics, to determine if blending of ruminal solids (which increases blank gas production) improves the rate of gas production kinetics, and to determine if preparing inoculum with warm buffer affects early fermentation.

Materials and Methods

Transistorized pressure transducers were used with a computer data acquisition system to continuously monitor pressure accumulation in sealed serum bottles with a head space for gas of about 48 ml after media is added. Approximately .1100 gm of material was fermented. Bottles containing the sample, a small Teflon-coated stir bar, 8 ml of media (6 ml when blended ruminal inoculum was used) were warmed in a water bath at 39 °C. Each was individually purged with CO_2 before .3 ml of reducing solution was added and bottles were lightly stoppered. At the time of inoculation, bottles were moved from the water bath, injected with about 2 ml of ruminal fluid (or 4.1 ml of blended ruminal inoculum), and the inoculum weight was recorded. Each bottle was stoppered, sealed with an aluminum crimp, immediately connected to a transducer using a 20-gauge needle. Gas pressure was recorded every 0.1 hr for at least 3 hr. Sample materials included glucose, purified starch and citrus pectin, an alfalfa hay standard, and 20 alfalfa genotypes.

Rumen contents (both liquid and solids) were collected in the morning (AM) and afternoon (PM) on three days from two lactating cows that were fed a mixed ration containing alfalfa silage, corn silage, high moisture corn, soybean meal and minerals. Cows were fed ad libitum with feed offered once in the morning of each day. Within 10 min after collection, the top 3-5 cm of solids were discarded and the remaining contents were strained through 2 layers of cheesecloth. In experiment 1, 50 gm of strained solids and 100 ml of strained ruminal fluid from each cow were combined and blended for 60 sec. The ruminal fluid and blended material were each strained through four layers of cheesecloth to obtain strained ruminal fluid (SRF) and blended ruminal fluid (BRF) that was used as inocula. Both SRF and BRF were also centrifuged at 500g for 5 min to produce centrifuged SRF (CSRF) and BRF (CBRF). In experiment 2, 50 ml of strained ruminal solids from each cow were combined with 220 ml of previously warmed and reduced in vitro media and blended for 60 sec.

Blended material and 100 ml of strained ruminal fluid from each cow were strained through four layers of cheesecloth to obtain blended ruminal media (BRM).

Results and Discussion

In experiment 1, SRF obtained a larger volume of fermentative gas production on samples than BRF (Table 1). Although centrifuging reduced the amount of gas produced by the blanks for both CSRF and CBRF, it also resulted in lower gas production by samples. The lower gas production by the BRF compared to SRF was surprising because in previous studies BRF had provided greater extents of fiber digestion when measured at longer fermentation. The dramatic depression in gas production when ruminal fluids were centrifuged was also unexpected. The low centrifugal force that was used could not have sedimented bacteria unless they were attached to particles. It is suspected that the reduced activity of centrifuged inocula may be due to temperature decreases during centrifugation. Temperature of these inocula were about 33 °C at the time of inoculation. Temperatures of BRF were also less than 39 °C at the time of inoculation due to cooling of the ruminal fluid during blending.

In experiment 2, ruminal solids were blended with warmed in vitro media that had been purged with CO_2 and reduced. It was hypothesized that blending of solids with warmed media would detach additional ruminal microorganisms and improve initial rates of gas production. As shown in Table 1, BRM resulted in slightly higher blank gas production, but also significantly increased gas production of samples. In both experiments, inocula collected in the afternoon had higher blank gas production, but the gas production of samples was higher in the PM in experiment 1 and lower in the PM in experiment 2. There were differences among days, but the day effect could be removed by using a standard hay or purified pectin as a covariate. In experiment 2 we observed an effect due to location in the incubator that is unexplained, but could be accounted for statistically.

Summary and Conclusion

It appears that both the source and method of preparing inoculum can affect in vitro gas production during early fermentation. To maximize the rate of gas production and ensure that the method used in the in vitro system is not limiting digestion kinetics, ruminal solids should be blended with warmed and reduced in vitro media to extract additional microorganisms. It is recommended that ruminal contents be collected from at least 2 and preferably 4 cows fed different diets and that strained ruminal fluid from these cows be mixed with warmed, reduced media that has been blended with ruminal solids and strained.

Table 1. Volume of fermentative gasses produced by blanks and samples when the ruminal fluid is strained (SRF) or blended with solids (BRF) and then centrifuged (CSRF or CBRF, respectively) in experiment 1 or when using SRF or strained ruminal fluid plus ruminal solids blended with in vitro media (BRM) in experiment 2.

Fermentation time	ml of CO_2 equivalent produced							
	Blanks				Sample average			
Experiment 1	SRF	CSRF	BRF	CBRF	SRF	CSRF	BRF	CBRF
1 hr	4.04	2.14	4.40	3.28	1.76	0.95	1.23	0.84
2 hr	5.26	2.28	5.06	3.52	4.38	2.76	3.60	2.52
3 hr	6.08	2.38	5.82	3.68	7.46	5.30	6.29	5.02
Experiment 2	SRF		BRM		SRF		BRM	
1 hr	3.32		3.52		1.60		2.68	
2 hr	4.06		4.72		3.91		5.47	
3 hr	4.60		5.52		6.53		8.27	